

- 1. A combination of substances, at least two of which exhibit amphipatic properties when contacted with a suitable liquid medium, said two substances differing in their solubility in this medium and said combination being capable of forming extended surfaces, especially membrane surfaces, in contact with said medium, such that molecules of an amphipatid third substance can associate with said surface, wherein said at least two substances are selected so that
- substance which is more soluble in said liquid medium than the other substance forms less extended surfaces than said other substance of the combination and -molecules of the third substance are more likely to associate with the extended surfaces formed by the other at least two substances combined than with an extended surface formed by said other, less soluble substance alone.
- 2. A combination of substances, at least two of which exhibit amphipatic properties when contacted with a suitable liquid medium, said two substances being capable of forming, at least when combined, an extended surface, especially a membrane surface, in contact with said medium, said surface carrying a net electric charge, such that molecules of a further amphipatic substance with a net electric charge can associate with said surface, and the net charge density of the surface and the net 20 charge of the amphipatic molecules associating with the surface have the same sign (both negative or both positive).
- A combination of substances, at least two of which exhibit amphipatic 3. properties when contacted with a suitable liquid medium said two substances differing 25 in their solubility in this medium and being capable of forming, at least when combined, extended surfaces, especially membrane surfaces, in contact with said medium, such that molecules of an amphipatic third substance can associate with said surfaces, said at least two substances being selected so that
- -the substance which is more soluble in said liquid medium than the other substance 30 forms less extended surfaces than said other substance of the combination,

WO 00/24377 PCT/EP98/06750

-molecules of the third substance are more likely to associate with the extended surfaces formed by the combination of the two substances than with an extended surface formed only by said other, less soluble substance, and

-the surfaces formed by the combined substances as well as the molecules of the third substance likely to associate with said surface, are both negatively charged or both positively charged.

4. A combination according to claim 1,2013,

characterised in that it comprises at least one amphipatic substance capable of self-aggregating to form an extended surface, which becomes more flexible when said substance is mixed with other combination components, especially with an amphipatic substance which is more soluble in the liquid medium than said self-aggregating substance, and especially where said two substances differ in solubility in the medium at least 10-fold, and preferably at least 100-fold.

15

20

10

5

5. A combination according to claims 1, 2 or 3,

characterised in that it comprises at least one amphipatic substance capable of self-aggregating to form an extended surface, and at least one amphiphatic substance which, when incorporated into said surface, supports an increased curvature of said surface, the concentration of said curvature-increasing substance being below 99% of the saturation concentration, or of that concentration above which the surface could not be formed, whichever is higher.

- 6. A combination according to claim 4 or 5;
- characterised in that the concentration of said more soluble or curvature-increasing substance amounts to at least 0.1 %, frequently to 1-80 %, more preferably to 10-60 %, and most preferably to 20-50 % of the relative concentration as defined in claim 5.
 - 7. A combination according to claim 5 or 6,
- characterised in that the surfaces have an average curvature (defined as the inverse average radius of the areas enclosed by the surfaces) corresponding to an average radius

between 15 nm and 5000 nm, often between 30 nm and 1000 nm, more often between 40 nm and 300 nm and most preferably between 50 nm and 150 nm.

8. A combination according to any one of claims 5 to 7;

characterised in that the surface is supported by a solid, especially by a supporting surface of suitable curvature or size

9. The combination of any pne of claims 2 through 8,

characterised in that the relative concentration of surface-related charged components is between 5 and 100 rel. mole-%, more preferably between 10 and 80 rel. mole-%, and most preferably between 20 and 60 rel. mole-% of the concentration of all surface-forming amphipats taken together.

10. Combination according to any one of claims 2 to 9,

15 **characterised in that** the average charge density on the surface is between 0.05 Cb m⁻² (Coulomb per square meter) and 0.5 Cb m⁻², preferably between 0.075 Cb m⁻² and 0.4 Cb m⁻² and particularly preferably between 0.10 Cb m⁻² and 0.35 Cb m⁻².

11. The combination of any one of claims 2 through 10,

characterised in that the concentration and the composition of background electrolyte, which preferably comprises mono or oligovalent ions, is chosen so as to maximise the positive effect of charge-charge interactions on the desired association and corresponds to ionic strength between I = 0.001 and I = 1, preferably between I = 0.02 and I = 0.5, and even more preferably between I = 0.1 and I = 0.3.

25

30

20

10

12. The combination of any one-of-claims-1-through 11,

characterised in that the substance which is less soluble in the liquid medium, and which preferably is the surface-building and/or charge carrying amphipatic substance in the system, is a lipid or lipid-like material, whereas the substance which is more soluble in the liquid medium, and preferably is the substance causing increased surface curvature, flexibility or adaptability and/or is the charge carrying substance, is a surfactant, or is identical with the third, associating substance.

62 lam 1

characterised in that it comprises arrangements of molecules in the form of minute fluid droplets suspended or dispersed in a liquid medium and surrounded by a membrane-like coating of one or several layers of at least two kinds or forms of self-aggregating amphiphilic substances, said at least two substances having an at least 10-fold, preferably an at least 100-fold difference in solubility in the preferably aqueous, liquid medium, such that the average diameter of homo-aggregates of the more soluble substance or of hetero-aggregates of both substances is smaller than the average diameter of homo-aggregates of the less soluble substance.

10

20

25

- 14. Combination according to any-one of the preceding claims, wherein the total content of all amphipats that can form a surface is between 0.01 and 30 weight-%, particularly between 0.1 and 15 weight-%, and most preferably between 1 and 10 weight-% of the total dry mass of the aggregates, especially if the combination is to be applied on or in the human or animal body.
 - characterised in that it contains at least one (bio)compatible polar or non-polar surface-supporting lipid as the substance which forms more extended surfaces, wherein the surfaces formed by the combination preferably have a bilayer structure.
 - 16. Combination according to claim 15, wherein said extended surfaceforming substance is a lipid or a lipoid from a biological source or a corresponding
 synthetic lipid, or is a modification of such a lipid, preferably a glyceride,
 glycerophospholipid, isoprenoidlipid, sphingolipid, steroid, sterine or sterol, a sulphuror carbohydrate-containing lipid, or any other lipid capable of forming bilayers, in
 particular a half-protonated fluid fatty acid, and preferably selected from
 phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols,
 phosphatidylinositols, phosphatidic acids, phosphatidylserines, sphingomyelins or
 sphingophospholipids, glycosphingolipids (e.g. it is a cerebroside,
 ceramidpolyhexoside, sulphatide, sphingoplasmalogene), gangliosides, or other
 glycolipids or synthetic lipids, in particular of the dioleoyl-, dilinoleyl-, dilinolenyl-,

10

15

20

25

30

WO 00/24377 PCT/EP98/06750

63

dilinolenoyl-, diarachidoyl-, diauroyl-, dimyristoyl-, dipalmitoyl-, distearoyl, or corresponding sphingosine derivative, or any other glycolipid or diacyl-, dialkenoyl-, or dialkyl-lipid.

Combination according to any of claims 12-through 16, wherein said 17. surfactant is a nonionic, a zwitterlonic, an anionic or a cationic surfactant, especially a long-chain fatty acid or alcohol, an alkyl-tri/di/methyl-ammonium salt, an alkylsulphate salt, a monovalent salt of cholate, deoxycholate, glycocholate, glycodeoxycholate, taurodeoxycholate, or taurocholate, an acyl- or alkanoyl-dimethyl-aminoxide, esp. a dodecyl- dimethyl-aminoxide, an alkyl- or alkanoyl-N-methylglucamide, N-alkyl-N,Ndimethylglycine, 3-(acyldimethylammonio)-alkanesulphonate, N-acyl-sulphobetaine, a polyethylen-glycol-octylphenyl ether esp. a nonaethylen-glycol-octylphenyl ether, a polyethylene-acyl ether, esp. a nonaethylen-dodecyl ether, a polyethyleneglycol-isoacyl ether, esp. a octaethyleneglycol-isotriflecyl ether, polyethylene-acyl ether, esp. octaethylenedodecyl ether, polyethyleneglykol-sorbitane-acyl ester, such as polyethylenglykol-20-monolaurate (Tween 20) or polyethylenglykol-20-sorbitanmonooleate (Tween 80), a polyhydroxyethylene-acyl ether, esp. polyhydroxyethylenelauryl, -myristoyl, -cetylstearyl, or -ole hyllether as in polyhydroxyethylen-4 or 6 or 8 or 10 or 12, etc. -lauryl ether (as in Brij serles), or in the corresponding ester, e.g. of polyhydroxyethylen-8-stearate (Myri 45), -laurate or -oleate type, or in polyethoxylated castor oil 40 (Cremophor EL), a sorbitane-monoalkylate (e.g. in Arlacel or Span), esp. sorbitane-monolaurate (Arlacel 20, Span 20), an acyl- or alkanoyl-N-methylglucamide, esp. in or decanoyl- or dodecanoyl-N-methylellucamide, an alkyl-sulphate (salt), e.g. in lauryl- or oleoyl-sulphate, sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium taurate, a fatty acid salt, such as sodium elaidate, sodium linoleate, sodium laurate, a lysophospholipid, such as n-octadecylene(=oleoyl)glycerophosphatidic acid, -phosphorylglycerol, on-phosphorylserine, n-acyl-, e.g. lauryl or oleoyl-glycero-phosphatidic acid, -phosphorylglycerol, or -phosphorylserine, ntetradecyl- glycero-phosphatidic acid, -phosphorylglycerol, or -phosphorylserine, a corresponding palmitoeloyl-, elaidoyl-, vaccenyl-lysophospholipid or a corresponding short-chain phospholipid, or else a surface-active polypeptide.

18. Combination according to any-one of claims 12 through 17; characterised in that the surface formed from the combination contains charged membrane components in the relative concentration range 1 to 80 mol-%, preferably 10 to 60 mol-% and most preferred between 30 and 50 mol-%.

5

10

15

20

25

- characterised in that a phosphatidylcholine and/or a phosphatidylglycerol is the surface-supporting substance and a lysophospholipid, such as lysophosphatidic acid or methylphosphatidic acid, lysophosphatidylglycerol, or lysophosphatidylcholine, or a partially N-methylated lysophosphatidylethanolamine, a monovalent salt of cholate, deoxycholate-, glycocholate, glycodedxycholate- or any other sufficiently polar sterol derivative, a laurate, myristate, palmitate, cleate, palmitoleate, elaidate or some other fatty acid salt and/or a Tween-, a Myrj- or a Brij-type, or else a Triton, a fatty-sulphonate or -sulphobetaine, -N-glucanide or -sorbitane (Arlacel or Span) surfactant is the substance less capable of forming the extended surface.
- 20. Combination according to one of claims 11 through 19, characterised in that the average radius of the areas enclosed by said extended surfaces is between 15 nm and 5000 nm, often between 30 nm and 1000 nm, more often between 40 nm and 300 nm and most preferably between 50 nm and 150 nm.
- 21. Combination according to anytone of the preceding claims, characterised in that the third substance, which can associate with the extended surface, comprises contains repeating subunits, especially in the form of chain molecules, such as oligomers or polymers, especially with an average molecular weight above 800 Daltons, preferably above 1000 Daltons and often even above 1500 Daltons.
- 22. Combination according to claim 21 characterised in that the third substance is of biological origin, and preferably is bioactive.

23. Combination according to any one of claims 1 through 22, characterised in that the third substance associates with the membrane-like extended surface, especially by inserting itself in the interface(s) between the membrane and the liquid medium in contact with said membrane.

5

10

15

20

25

- 24. Combination according to any-one of claims 1 to 23, wherein the content of chain molecules corresponding to said third substance, is between 0.001 and 50 rel. % compared to the mass of adsorbent surface and often is between 0.1 and 35 rel. %, more preferably is between 0.5 and 25 rel. %, and most suitably is between 1 and 20 rel. %, whereby the specific ratio value is likely to decrease with increasing molar mass of said chain molecules.
- 25. Combination according to any one of claims 21 through 24, wherein chain molecule is a protein, and at least a part of said molecule is associated with the surface, provided that such part has at least three segments or functional groups with a propensity to bind to said surface.
 - 26. Combination according to any one of claims 21 through 24, characterised in that said chain molecules belong to the class of polynucleotides, such as DNA or RNA, in the natural form or after chemical, biochemical, or genetic modification.
 - 27. Combination according to any one of claims 21 through 24, characterised in that said chain molecules belong to the class of polysaccharides with at least partial propensity to interact with the surface either in the natural form or after some chemical, biochemical, or genetic modification.
 - 28. Combination according to any one of claims 21 through 27; wherein the chain molecule can act as an adrenocorticostaticum, a β-adrenolyticum, an androgen or antiandrogen, antiparasiticum, anabolicum, anaestheticum or analgesicum, analepticum, antiallergicum, antiarrhythmicum, antiarteroscleroticum, antiasthmaticum and/or bronchospasmolyticum, antibioticum, antidrepressivum and/or antipsychoticum,

66 antidiabeticum, an antidot, antiemeticum, antiepilepticum, antifibrinolyticum, anticonvulsivum, anticholinergicum, enzyme, a coenzyme or corresponding inhibitor, an antihistaminicum, antihypertonicum, a biological inhibitor of drug activity, an antihypotonicum, anticoagulant, antimycoticum, antimyasthenicum, agent against Morbus Parkinson or Morbus Alzheimer, an antiphlogisticum, antipyreticum, 5 antirheumaticum, antisepticum, a respiratory analepticum or a respiratory stimulant, a broncholyticum, cardiotonicum, chemotherapeuticum, a coronary dilatator, a cytostaticum, a diureticum, a ganglium-blocker, a glucocorticoid, an antiflew agent, a haemostaticum, hypnoticum, an immunoglobuline or its fragment or any other immunologically active substance, a bioactive carbohydrate(derivative), a contraceptive, 10 an anti-migraine agent, a mineralo-corticoid, a morphine-antagonist, a muscle relaxant, a narcoticum, a neurotherapeuticum, a neurolepticum, a neurotransmitter or its antagonist, a peptide(derivative), and optifalmicum, (para)-sympaticomimeticum or (para)sympathicolyticum, a protein(derivative), a psoriasis/neurodermitis drug, a mydriaticum, a psychostimulant, rhindlogicum, any sleep-inducing agent or its 15 antagonist, a sedating agent, a spasmolyticum, tuberculostaticum, urologicum, a vasoconstrictor or vasodilatator, a virus aticum or any of the wound-healing substances, or any combination of aforesaid agents.

29. Combination according to any one of the preceding claims, wherein said third substance chain molecule or agent is a growth modulating substance.

30. Combination according to any of the preceding claims, wherein said third substance agent has immunomodulating properties, including antibodies, cytokines, lymphokines, chemokines and correspondingly active parts of plants, bacteria, viruses, pathogens, or else immunogens, or parts or modifications of any of these.

31. Combination according to any-one of the preceding claims, wherein said third substance agent is an enzyme, a co-enzyme or some other kind of bio-catalyst.

25

32. Combination according to any one of the preceding claims, wherein said third substance agent is a recognition molecule, including inter alia adherins, antibodies,

catenins, selectins, chaperones, or parts thereof.

WO 00/24377

5

10

15

20

25

30

PCT/EP98/06750

33. Combination according to any one of the preceding claims, wherein said agent is a hormone, especially insulin.

34. Combination according to any one of the preceding claims; characterised in that it contains 1 through to 500 I.U. insulin/mL, in particular between 20 and 400 I.U. insulin/mL and most preferred between 50 and 250 I.U. insulin/mL, preferably of human recombinant or humanised type.

35. Combination according to any one of the preceding claims, characterised in that it contains between 0.01 mg and 20 mg interleukin/mL, in particular between 0.1 and 15 mg and most preferred between 1 and 10 mg interleukin/mL, said interleukin being suitable for the use in humans or animals, including IL-2, IL-4, IL-8, IL-10, IL-12 if necessary after a final dilution to reach the practically desirable drug concentration lange.

36. Combination according to any one of the preceding claims, characterised in that it contains up to 20 relative wt-% interferon, in particular between 0.1 and 15 mg interferon/mL and most preferred between 1 and 10 mg interferon/mL, said IF being suitable for the use in humans or animals, including but not restricted to IF alpha, beta and gamma, can be used, if necessary after a final dilution that brings the drug concentration into practically preferred concentration range.

37. Combination according to any one of the preceding claims, characterised in that it contains up to 25 mg nerve growth factor (NGF) / mL suspension or up to 25 relative w-% of NGF as an agent, especially 0.1-15 rel. w-% protein and most preferred between 1 and 10 rel. wt-% NGF, preferably human recombinant NGF and, if needed, diluted before use.

- characterised in that the suspension contains up to 25 mg of immunoglobulin(lg)/mL suspension or up to 25 w-% of Ig relative to total lipid, preferably with 0.1 rel. w-% to 15 rel. w-% protein and most advisable with 1 rel. w-% to 10 rel w-% immunoglobulin, whereby the agent is used in the form of an intact antibody, part of it, or a biologically acceptable and active modification thereof.
- 39. A method of preparing a formulation of an active agent, especially a biologically, cosmetically and/or pharmaceutically active agent,
- 10 characterised by the steps of
 selecting at least two amphipatic

WO 00/24377

5

- selecting at least two amphipatic substances, which differ in their solubility in a suitable liquid medium, such substances being capable of forming an extended surface, especially a membrane surface, at least when combined in contact with said medium,
- such that an extended surface formed by the combination of substances is capable of attracting and associating with the active agent to a greater extent than the surface formed only from the substance which is less soluble in the liquid medium and forms more extended surfaces than the other substance alone.
 - 40. The method according to claim 39,
- characterised in that the combination of surface-forming substances is generated by filtration, pressure change or mechanical homogenisation, shaking, stirring, mixing, or by means of any other controlled mechanical fragmentation, in the presence of agent molecules.
- 25 41. The method according to claim 39, in which the selected combination of surface forming substances is permitted to adsorb to, or in some other way is brought into permanent contact with, (a) suitable supporting solid surface(s), and then with the liquid medium by adding one substance after another or several at a time, whereby at least one of the later surface-forming steps is carried out in the presence of the agent that subsequently associates with the solid-supported surface.

15

20

preformed surfaces.

42. The method according to claim 38, characterised in that the adsorbing surfaces or their precursors, whether suspended in a liquid medium or supported by a solid, are first prepared by steps which may include sequential mixing of the surface forming molecules, and the associating molecules are then added and permitted to associate with the said surfaces, if necessary assisted by agitation, mixing or incubation, provided that such treatment does not break-up the

- 43. The method according to claims 39 through 42,

 characterised in that the surfaces with which the agent molecules associate correspond to any one of claims 1 through 37.
 - 44. The method according to claims 39 through 43, characterised in that the liquid meaning suspension characteristics correspond to any one of claims 1 to 37.
 - 45. A method for the preparation of a formulation for non-invasive application of various agents, such as anti-diabetic agents, growth factors, immunomodulators, enzymes, recognition molecules, etc., or adrenocorticostatica, adrenolitica, etc., wherein surfaces capable of associating with said agent molecules are formed from at least one amphiphilic substance, at least one hydrophilic fluid, at least one edge active substance or surfactant, at least one agent and, in case, other customary ingredients, which together form said formulation.
- 25 46. The method of claim 45,

 characterised in that at least one edge-active substance or a surfactant, at least one
 amphiphilic substance, at least one hydrophilic fluid and the agent are separately mixed
 and, if required, dissolved to form a solution, the resulting mixtures or solutions then
 being combined to subsequently induce, preferably by action of mechanical energy, the
 formation of the entities which associate with the agent molecules.

characterised in that said amphibilic substances are either used as such, or dissolved in a physiologically compatible polar fluid, which may be water or miscible with water, or in a solvation-mediating agent, together with a polar solution.

5

48. The method as claimed in claim 47, wherein the said polar solution contains at least one edge-active substance or a surfactant.

49. The method according to any one of claims 45 through to 48; characterised in that the formation of said surfaces is induced by substance addition into a fluid phase, evaporation from a reverse phase, by injection or dialysis, if needed with the aid of mechanical stress, such as shaking, stirring, vibrating, homogenising, ultrasonication, shear, freezing and thaving, or filtration using convenient driving pressure.

15

20

10

characterised in that the formation of said surfaces is induced by filtration, the filtering material having pores sizes between 0.01 μ m and 0.8 μ m, preferably between 0.02 μ m and 0.3 μ m, and most preferably between 0.05 μ m and 0.15 μ m, whereby several filters may be used sequentially or in parallel.

51. The method according to any one of claims 45 through 50, characterised in that said agents and carriers are made to associate, at least partly, after formation of the adsorbing surface.

25

52. The method according to any one of the claims 45 through to 51, characterised in that said surfaces, with which the agent molecules associate, are prepared just before the application of the formulation, if convenient from a suitable concentrate or a lyophylisate.

10

53. Use of a combination of substances in accordance with any one of the preceding claims, for the preparation of drug carriers, drug depots, or for any other kind of medicinal or biological application.

Use of a combination of substances in accordance with any one of the preceding claims, in bioengineering or for genetic manipulations.

Use of a combination of substances in accordance with any one of the preceding claims; in separation technology, for (bio)processing or for diagnostic purposes.

56. Use of a combination of substances in accordance with any one of the preceding claims to stabilise surface associating molecules, especially chain molecules, that are at least partially amphipatic, such as (derivatised) proteins, polypeptides, polynucleotides, or polysaccharides and/or in catalysing processes which involve such molecules in the surface-associated state.

57. Use of a combination of substances in accordance with any one of the preceding claims to affect the kinetics and/or the reversibility of association or dissociation between said surface-associating molecules and a complex, adaptable surface, whereby the higher surface charge density and/or greater surface softness and/or surface defect density speeds up the association, or the corresponding reduction slows down the rate of association or else induces partial molecular dissociation.

add 8¹7